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EDITORIAL COMMENTARY

Herpesviruses and the Hayflick Limit In Vivo

AQ1 **Paul D. Griffiths**

EAB Member - University College, London, United Kingdom

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How should we assess the medical significance of a virus that infects many persons but seems innocuous? Take cytomegalovirus (CMV) as an example; most of the world's adults are seropositive without any symptoms they can attribute to this infection; so should we dismiss CMV as an unimportant pathogen? Before doing so, we should acknowledge that subjective assessments of disease associations reported by patients can be misleading. For example, persons who smoke cigarettes may appear to be well at the time they are interviewed and those with primary human immunodeficiency virus (HIV) infection are often without overt symptoms. In both cases, long-term follow-up was required to determine the full clinical significance of these conditions. What has been revealed by the equivalent studies for CMV?

Stored samples from the US National Health and Nutrition Examination Survey (NHANES) cohort III (1988–1994) were tested for CMV immunoglobulin (Ig) G antibodies, and findings were epidemiological[AQ2] typical, with 67% of the adult population seropositive [1]. Nevertheless, linkage to death certification showed that, after a median 13.7-year follow-up from the initial serologic assessment, the mortality rate was significantly higher in those who were CMV seropositive than in those who were seronegative. The effect was attenuated by controlling for risk factors, including diabetes, smoking, and obesity, but it remained significant, with a relative hazard for death of 1.19 (that is, a 19% relative increase in mortality). This strikingly large effect prompted investigators from a second large population cohort in the United Kingdom to retrieve their serum samples and test for CMV IgG. They reported that, after a mean follow-up of 14.3 years, the 59% of the population who were CMV seropositive had a very similar relative hazard of 1.16 for death, after controlling for established risk factors [2]. In both studies, excess deaths were attributed to cardiovascular disease and to cancer [1, 2] Is it plausible that this association is causal? Are there any known mechanisms that might link asymptomatic CMV infection to an excess of deaths? Is there evidence of a similar association with other herpesviruses, or is CMV unique among that virus family? Remarkably, there several biologic phenomena that might explain such an association.

CMV persists lifelong by hiding from the immune system [3]. It does this by expressing its immune evasion genes so that infected cells remain invisible to the immune system, allowing CMV to persist in sanctuary sites within the body [4, 5]. Some immune evasion genes provide decoy signals to natural killer (NK) cells and macrophages equivalent to those on healthy host cells, while others interfere with the stress signals that would otherwise stimulate an NK attack on cells whose biochemistry has been hijacked by a virus [4]. Many of these signals work through the HLA class I system, either by interfering with normal display of these proteins at the cell surface or by increasing display of inhibitory molecules, such as HLA-E [6]. The net effect is that the number of T cells that become committed to searching for CMV sanctuary sites increases as we age and acquire the TEMRA phenotype (T-effector cells reexpressing the RA marker classically associated with naive rather than memory cells) [7–9]. Likewise, the surface proteins expressed by NK cells become changed to express phenotypic markers that can be detected readily [10–12].

It is not clear whether all these changes are driven by a small number of sanctuary sites established during primary infection or whether periodic reactivations of latent virus or reinfections with different strains of CMV are also required. Nevertheless, after years or decades of clinically silent CMV infection, the immune system commits more resources to CMV than to all the other viruses an elderly person has encountered in his or her life [13]. This has 3 major potential effects: the excess of immune cells could contribute to chronic inflammatory diseases such as atherosclerosis; the relative lack of naive T cells could impair the ability of elderly persons to respond to new antigens of pathogens, including those found in vaccines; and the normal immune surveillance function of these cells could be impaired. Death would be attributed to atherosclerosis in the first scenario, to influenza or pneumococcal disease in the second scenario and to cancer in the third scenario, without recognizing the contribution made by underlying CMV infection. Put bluntly, physicians caring for elderly persons may currently be writing incomplete death certificates

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Correspondence: P. D. Griffiths, EAB Member - University College, London, UK (p.griffiths@ucl.ac.uk)

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that provide the overt, distal cause of death without recording the covert, proximal long-term contribution made by CMV.

In this issue of *The Journal of Infectious Diseases*, Dowd et al [14] discuss yet another potential mechanism. In a longitudinal study design involving 3-year follow-up in 400 healthy, middle-aged civil servants, the change in telomere length of peripheral blood mononuclear cells was described and related to the presence at baseline of IgG antibodies against 4 herpesviruses. Three of these (CMV, herpes simplex virus, human herpesvirus 6) were associated with attrition of telomere lengths. The strongest effect came from CMV, with evidence of an additive effect from multiple herpesviruses. The effect size was large, equivalent to 12 chronological years of normal aging in those who were CMV seropositive. The effect of shortened telomeres will be familiar to laboratory researchers in terms of the number of cell divisions that are possible in vitro before cells become senescent (Hayflick limit) [15]. It is tempting to speculate that an equivalent process in vivo would lead to progressive dysfunction of cells in organs and in the immune system, thereby contributing to immunosenescence. Although comparison of telomere lengths from cells of individuals of different ages does not support that simple conclusion, telomere shortening is, nevertheless, taken to represent a general process of aging within an individual [16, 17].

Thus, we have multiple plausible mechanisms linking CMV, and perhaps other herpesviruses, with causes of death. It should be noted that the various phenomena studied so far overlap. For example TEMRA T cells have shortened telomeres, chronic stress or inflammation can shorten telomeres, and stress is known to trigger reactivation of herpesviruses, as illustrated dramatically by studies of astronauts [9, 17–19]. Assuming that some of these effects turn out to be truly caused by CMV and other herpesviruses, what action could be taken? Universal immunization with a vaccine able to protect individuals against primary infection would be effective, but only after many decades [20]. As probes of pathogenesis, vaccines are specific for each virus and could be used for immunotherapy to determine if boosting of immunity controls each virus and its putative effects on immunosenescence. Immunotherapy for herpesviruses has been reported for varicella-zoster virus and CMV [21–23].

In contrast, antiviral drugs give a more immediate inhibition of virus replication, and may have pan-herpesvirus activity. Clinical trials of these drugs should, therefore, investigate effects on all herpesviruses, not just on the one being targeted. A course of chemotherapy could be given as part of an investigational randomized controlled trial. It would be unrealistic to ask grant-giving bodies to support the long-term follow-up required to examine survival as an end point, but reversal of abnormal immunologic indices attributed to CMV or reversal of telomere attrition would represent good first steps. Accordingly, the abundance of activated CD8 T cells was significantly reduced in a double-blind, randomized placebo-controlled trial of valganciclovir in HIV-positive patients [24]. To expand on the findings reported by Dowd et al [24] in this issue, the enzyme telomerase could be activated to increase telomere lengths, but caution is required, because this enzymatic activity is associated with the undesirable life extension found in cancer cells.

The challenge now is for investigators to determine whether the various reported associations with herpesviruses are causal and whether their effects can be ameliorated through the repurposing of existing medical interventions. The prize is none other than the potential slowing down of the aging process that has been taken to be part of normal life, but might be reclassified as having a pathologic component driven by silent herpesvirus infections.

Notes

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